

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION

ATTORNEY DKT NO: 5233.012.NPUS00

OF: GARNER

CONFIRMATION NO.: 5154

SERIAL NO. 10/711,155

GROUP ART UNIT: 1634

FILED: AUGUST 27, 2004

EXAMINER: SHAW, AMANDA MARIE

TITLE: METHODS FOR DETECTING AND QUANTIFYING  
SPECIFIC PROBIOTIC MICROORGANISMS IN  
ANIMAL FEED

AMENDMENT AND REPLY UNDER 37 C.F.R. §1.111

Sir,

In response to the non-final office action mailed on April 14, 2006, please enter the following amendments to the claims, and reconsider the application in view of these amendments and Applicant's remarks below.

- (1) Amendments to the claims are reflected in the listing which begins on page 2 of this paper.
- (2) Remarks begin on page 8 of this paper.

**AMENDMENTS TO THE CLAIMS**

The Listing of claims below replaces all prior versions, and listings, of claims in the application.

1. (currently amended) ~~A method of quantifying a presence of a specific kind of probiotic microorganism in a sample of animal feed, said method comprising: (a) culturing the sample under conditions suitable for growth of cultures of the specific kind of probiotic microorganism; (b) using at least one oligonucleotide to detect the presence or absence of the specific kind of probiotic microorganism in respective portions of the cultured sample; and (c) quantifying the presence of the specific kind of probiotic microorganism in the sample of material from the detected presence or absence of the specific kind of probiotic microorganism in the respective portions of the cultured sample~~

A method for assessing the relative quantity of a viable microorganism of interest that is present and has been previously applied to a food product in the course of microbially treating the food product, said method comprising:

obtaining a liquid suspension sample comprising different microorganisms removed from a microbial-treated food product and which includes a substantial entirety of a previously applied and viable microorganism of interest from a known quantity of the microbial-treated food product and in which the different microorganisms are suspended in a liquid recovery media of known quantity;

preparing a series of progressively dilute test samples by combining portions of the liquid suspension sample with a dilution liquid;

incubating the series of progressively dilute test samples for a predetermined period of time under conditions conducive to growth of the microorganism of interest;

conducting a PCR analysis on the series of progressively dilute test samples; and

utilizing an estimation model to determine the concentration of the viable microorganism of interest present on the food product based on results of the PCR analysis.

2. (currently amended) The method as claimed in claim 1, wherein said food product is a sample of animal feed and said method ~~which~~ includes taking the sample of animal feed from a feedpile and transporting the sample to a testing lab in such a way that the sample of the animal feed at the testing laboratory is representative of the condition of the animal feed when the animal feed is to be consumed by animals.

3. (currently amended) The method as claimed in claim 1, wherein said food product is a sample of animal feed and said method ~~which~~ includes taking the sample of animal feed from a feedpile at a location where the animal feed is to be consumed by animals.

4-6 (canceled)

7. (currently amended) The method as claimed in claim 1, wherein said at least one ~~oligonucleotide~~ oligonucleotide hybridizes with a nucleic acid sequence that is indicative of a species of the specific kind of microorganism.

8. (canceled)

9. (currently amended) The method of claim 1, wherein series of progressively dilute test samples ~~the sample is cultured by dividing the sample~~ are divided into multiple portions and after incubating ~~culturing~~ each portion, ~~and wherein the PCR analysis detects the presence or absence of the specific kind of microorganism of interest is detected~~ in each ~~cultured~~ incubated portion.

10. (currently amended) The method as claimed in claim 9, wherein the ~~sample is~~ series of progressively dilute test samples are divided into the multiple portions by diluting the test sample samples and dividing the diluted sample into the multiple portions.

11. (currently amended) The method as claimed in claim 9, wherein the series of progressively dilute test samples ~~sample is~~ are divided into multiple portions by mixing the sample with liquid to produce a fluid mixture, and dividing the fluid mixture into the multiple portions.

12. (currently amended) The method as claimed in claim 1, wherein the PCR analysis comprises the using ~~of~~ at least one oligonucleotide to detect the presence or absence of the ~~specific kind of probiotic microorganism of interest~~ in respective portions of the ~~cultured~~ incubated test samples ~~sample~~ and wherein said PCR analysis includes detecting the presence or absence of a product of hybridization of said at least one oligonucleotide with a nucleic acid sequence that is indicative of the ~~specific kind of probiotic microorganism of interest~~.

13. (currently amended) The method as claimed in claim 1, wherein the PCR analysis comprises ~~the using of at least one oligonucleotide to detect the presence or absence of the specific kind of probiotic microorganism in respective portions of the cultured sample~~ includes using using two oligonucleotide primers that induce a polymerase chain reaction in the presence of nuclear material of the ~~specific kind of probiotic microorganism of interest~~, and detecting the presence or absence of a product of the polymerase chain reaction of the two oligonucleotide primers in the presence of the nuclear material of the ~~specific kind of probiotic microorganism of interest~~.

14. (currently amended) The method as claimed in claim 13, wherein one of the ~~oligonucleotide~~ oligonucleotide primers hybridizes with a nucleic acid sequence indicative of the genus of the ~~specific kind of microorganism of interest~~, and another of the ~~oligonucleotide~~ oligonucleotide primers hybridizes with a nucleic acid sequence indicative of the species of the ~~specific kind of probiotic microorganism of interest~~.

15. (currently amended) The method as claimed in claim 13, wherein the detecting of the presence or absence of a product of the polymerase chain reaction of the two oligonucleotide primers in the presence of the nuclear material of the ~~specific kind of probiotic microorganism~~ of interest includes performing electrophoresis of polymerase chain reaction products to detect a reaction product having a characteristic molecular length indicative of a polymerase chain reaction of the two oligonucleotide primers in the presence of the nuclear material of ~~the specific kind of probiotic microorganism~~ of interest.

16. (currently amended) The method as claimed in claim 1, wherein the ~~presence of the specific kind of probiotic microorganism in the sample of material is quantified in terms of a~~ the estimation model for determining the concentration of the viable microorganism of interest is a most probable number method of the ~~specific kind of probiotic microorganism~~.

17-36 (canceled)

37. (new) The method of claim 1 wherein the microorganism of interest is a probiotic organism.

38. (new) The method of claim 37 wherein the microorganism of interest is selected from the group consisting of Bacillus subtilis, Bifidobacterium adolescentis, Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium thermophilum, Lactobacillus acidophilus, Lactobacillus agilis, Lactobacillus alactosus, Lactobacillus alimentarius, Lactobacillus amylophilus, Lactobacillus amylovorus, Lactobacillus animalis, Lactobacillus batatas, Lactobacillus bavaricus, Lactobacillus bifementans, Lactobacillus bifidus, Lactobacillus brevis, Lactobacillus buchnerii, Lactobacillus bulgaricus,

*Lactobacillus cateniformis*, *Lactobacillus casei*, *Lactobacillus cellobiosus*,  
*Lactobacillus collinoides*, *Lactobacillus confusus*, *Lactobacillus coprophilus*,  
*Lactobacillus coryniformis*, *Lactobacillus corynoides*, *Lactobacillus crispatus*,  
*Lactobacillus curvatus*, *Lactobacillus delbrueckii*, *Lactobacillus desidiosus*,  
*Lactobacillus divergens*, *Lactobacillus enterii*, *Lactobacillus farciminis*,  
*Lactobacillus fermentum*, *Lactobacillus frigidus*, *Lactobacillus fructivorans*,  
*Lactobacillus fructosus*, *Lactobacillus gasseri*, *Lactobacillus halotolerans*,  
*Lactobacillus helveticus*, *Lactobacillus heterohiochii*, *Lactobacillus hilgardii*,  
*Lactobacillus hordniae*, *Lactobacillus inulinus*, *Lactobacillus jensenii*,  
*Lactobacillus jugurti*, *Lactobacillus kandleri*, *Lactobacillus kefir*, *Lactobacillus*  
*lactis*, *Lactobacillus leichmannii*, *Lactobacillus lindneri*, *Lactobacillus*  
*malefermentans*, *Lactobacillus mali*, *Lactobacillus maltaromicus*, *Lactobacillus*  
*minor*, *Lactobacillus minutus*, *Lactobacillus mobilis*, *Lactobacillus murinus*,  
*Lactobacillus pentosus*, *Lactobacillus plantarum*, *Lactobacillus pseudoplanarum*,  
*Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus rogosa*,  
*Lactobacillus tolerans*, *Lactobacillus torquens*, *Lactobacillus ruminis*,  
*Lactobacillus sake*, *Lactobacillus salivarius*, *Lactobacillus sanfrancisco*,  
*Lactobacillus sharpeae*, *Lactobacillus trichodes*, *Lactobacillus vaccinoferus*,  
*Lactobacillus viridescens*, *Lactobacillus vitulinus*, *Lactobacillus xylophilus*,  
*Lactobacillus yamanashiensis*, *Lactobacillus zeae*, *Pediococcus acidilactici*,  
*Pediococcus pentosaceus*, *Streptococcus cremoris*, *Streptococcus discetylactis*,  
*Streptococcus faecium*, *Streptococcus intermedius*, *Streptococcus lactis*,  
*Streptococcus thermophilus*, and *Escherichia coli*. Another group of lactate  
utilizing microorganisms include *Propionibacterium freudenreichii*,  
*Propionibacterium shermanii*, *Propionibacterium jensenii*, *Propionibacterium*  
*acidipropionici*, *Propionibacterium thoenii*, *Propionibacterium*, *Megasphaera*  
*elsdenii*, *Selenomonas ruminantium*, and *Peptostreptococcus asaccharolyticus*.

39. (new) The method as claimed in claim 38, wherein the specific kind of probiotic microorganism is a species of *Lactobacillus*.

40. (new) The method as claimed in claim 38, wherein the specific kind of probiotic microorganism is *Lactobacillus acidophilus*.

41. (new) The method as claimed in claim 38, wherein the specific kind of probiotic microorganism is *Lactobacillus* LA-51.